REMARKS

The Examiner's action includes a rejection of all of the pending claims in the application except claim 55 (claims 1 to 14, 17 to 32, 53, and 54). Claim 55 has been objected to as depending on a rejected base claim. The action includes rejections under 35 U.S.C. §102(b), §103, and 35 U.S.C. §112, first and second paragraphs. Reconsideration of the allowability of the present application is requested respectfully.

Claims 1 to 14, 17 to 32, 53, and 54 were acted upon by the Examiner in the Office Action dated December 3, 2001. Amendments have been made to claims 1, 2, 4, 5, 7, 12, 13, 27 to 29, 31, and 53. No claims have been cancelled.

Applicants have presented new claim 56 which is based on claim 55 and which includes the relevant recitations of claim 4 upon which claim 55 depends. Accordingly, Claims 1 to 14, 17 to 32, and 53 to 56 are presented for examination. Applicants have presented the proposed amendments to put the claims in better form for appeal and/or to respond to the Examiner's requirements as to the form of the claims.

In response to the Examiner's Office Action dated December 3, 2001, Applicants respectfully traverse the Examiner's rejection of Claims 1 to 14, 17 to 32, 53, and 54.

1. The §102(b) Rejections

A. The Examiner has rejected claims 1 to 14, 17, 19, 20, and 26 as anticipated by Brant et al. (GenBank Accession No. T51330).

Claims 1 to 6, 8 to 14, 17, 19, 20 and 26, as amended, recite plant (PNHX) Na^+/H^+ transporters and fragments of a plant polypeptide having Na^+/H^+ transporter activity. Support for this amendment may be found on page 20, lines 7 to 8, and page 23, lines 17 to 19 of the application. As Brant et al. discloses a human Na^+/H^+ transporter and the rejected claims define a plant Na^+/H^+ transporter, claims 1 to 6, 8 to 14, 17, 19, 20, and 26 are not anticipated by Brant et al.

The basis of the rejection of claim 7 in view of Brant et al. is unclear to Applicants. Claim 7 teaches a nucleic acid isolated from *Arabidopsis thaliana* encoding a Na^+/H^+

transporter that can be used to provide salt tolerance. Brant et al. discloses a human Na^+/H^+ transporter. Thus, claim 7 is not anticipated by Brant et al.

B. The Examiner has rejected claims 1 to 14, 17, 19, 20, and 26 as anticipated by Sumitomo Sieyaku KK (GenBank Accession No. Q51524; hereafter "Sieyaku").

Claims 1 to 6, 8 to 14, 17, 19, 20 and 26, as amended, recite plant (PNHX) Na^+/H^+ transporters and fragments of a plant polypeptide having Na^+/H^+ transporter activity. Support for this amendment may be found on page 20, lines 7 to 8, and page 23, lines 17 to 19 of the application. As Sieyaku discloses a rabbit Na^+/H^+ transporter and the rejected claims define a plant Na^+/H^+ transporter, claims 1 to 6, 8 to 14, 17, 19, 20, and 26 are not anticipated by Sieyaku.

The basis of the rejection of claim 7 in view of Sieyaku is unclear to Applicants. Claim 7 teaches a nucleic acid isolated from *Arabidopsis thaliana* encoding a Na^+/H^+ transporter that can be used to provide salt tolerance. Sieyaku discloses a rabbit Na^+/H^+ transporter. Thus, claim 7 is not anticipated by Sieyaku.

C. The Examiner has rejected claims 2 to 14, 17, 19, 20, and 26 as anticipated by Dante et al. (GenBank Accession No. AF007271).

Claims 2 to 14, 17, 19, 20, and 26, as amended, recite "wherein said nucleic acid molecule is not the sequence of the gene A_TM021B04.4 or complementary to all of the sequence of the gene A_TM021B04.4". The gene A_TM021B04.4 is defined as the complement of nucleotides 65405 to 68024 of GenBank Accession No. AF007271 (see page 6 of Dante et al.; copy of pages 1, 6, 26, and 27 enclosed). In the Office Action of December 3, 2001, the Examiner provided a copy of a sequence alignment which identifies the sequence and sequence numbering of the A_TM021B04.4 gene. Support for this amendment can be found on page 21, lines 15 to 22, of the application, specifically the sentence on page 21, bridging lines 17 to 18, which states, "A comparison of this full length cDNA with the *Arabidopsis* genome sequence (A_TM021B04.4) revealed the presence of 13 introns and 14 exons". Because Applicants have made explicit reference to A_TM021B04.4 and have noted the homology between the nucleotide sequence of A_TM021B04.4 and the nucleotide sequence of AtNHX1 (SEQ ID NO:1), addition of claim language distinguishing the claimed

invention from A_TM021B04.4 is fully supported by the specification. By specifically distinguishing the sequences claimed in the present invention from the sequence of A_TM021B04.4, Applicants submit the rejection based on Dante et al. should be withdrawn.

D. The Examiner rejected claims 1 to 3, 5 to 14, 17 to 20, 26, and 31 as anticipated by Hahnenberger et al. (*Proc. Natl. Acad. Sci U.S.A.* (1996) 93:5031-5036).

Claims 1 to 3, 5, 6, 8 to 14, 17 to 20, 26, and 31, as amended, recite plant (PNHX) Na^+/H^+ transporters and fragments of a plant polypeptide having Na^+/H^+ transporter activity. Support for this amendment may be found on page 20, lines 7 to 8, and page 23, lines 17 to 19 of the application. As Hahnenberger et al. discloses a yeast Na^+/H^+ transporter and the rejected claims define a plant Na^+/H^+ transporter, claims 1 to 3, 5, 6, 8 to 14, 17 to 20, 26, and 31 are not anticipated by Hahnenberger et al.

The basis of the rejection of claim 7 in view of Hahnenberger et al. is unclear to Applicants. Claim 7 teaches a nucleic acid isolated from *Arabidopsis thaliana* encoding a Na^+/H^+ transporter that can be used to provide salt tolerance. Hahnenberger et al. discloses a yeast Na^+/H^+ transporter. Thus, claim 7 is not anticipated by Hahnenberger et al.

E. The Examiner has rejected claims 1 to 3, 5 to 14, 17 to 24, 26 to 32, 53, and 54 as anticipated by Young et al. (WO 91/06651).

Claims 1 to 3, 5, 6, 8 to 14, 17 to 20, 26, and 31, as amended, recite plant (PNHX) Na^+/H^+ transporters and fragments of a plant polypeptide having Na^+/H^+ transporter activity. Support for this amendment may be found on page 20, lines 7 to 8, and page 23, lines 17 to 19 of the application. As Young et al. discloses a yeast Na^+/H^+ transporter and the rejected claims define a plant Na^+/H^+ transporter, claims 1 to 3, 5, 6, 8 to 14, 17 to 20, 26, and 31 are not anticipated by Young et al.

The basis of the rejection of claim 7 in view of Young et al. is unclear to Applicants. Claim 7 teaches a nucleic acid isolated from *Arabidopsis thaliana* encoding a Na^+/H^+ transporter that can be used to provide salt tolerance. Young et al. discloses a yeast Na^+/H^+ transporter. Thus, claim 7 is not anticipated by Young et al.

In view of the above arguments, Applicants request respectfully that the Examiner reconsider and withdraw the §102(b) rejection of Claims 1 to 14, 17 to 24, 26 to 32, 53, and 54.

II. The §103(a) Rejection

The Examiner has alleged that claims 1 to 3, 5 to 14, 17 to 32, and 53 to 54 are unpatentable over Young et al. in view of Gordon-Kamm et al. (*Plant Cell* (1990), 2:603-618).

Claims 1 to 3, 5 to 14, 17 to 32, and 53 to 54, as amended, recite plant (PNHX) Na^+/H^+ transporters and fragments of a plant polypeptide having Na^+/H^+ transporter activity. Support for this amendment may be found on page 20, lines 7 to 8, and page 23, lines 17 to 19 of the application.

Young et al. teach a yeast Na^+/H^+ transporter. There is no disclosure in Young et al. regarding plant Na^+/H^+ transporters. Gordon-Kamm et al. does not teach Na^+/H^+ transporters.

Applicants submit that a *prima facie* case for a §103 rejection cannot be established for the present invention as presently amended. As indicated in the MPEP §2143.03, to establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 U.S.P.Q. 580 (CCPA 1974). The Examiner has indicated that Young et al. teach tobacco and *Arabidopsis* plants transformed with a gene encoding the Na^+/H^+ transporter sod2. However, neither Young et al. nor Gordon-Kamm et al. teach or suggest introducing a plant Na^+/H^+ transporter into plants. In fact, there is no teaching in Young et al. or Gordon-Kamm et al. regarding plant Na^+/H^+ transporters. Accordingly, all the claim limitations are not taught or suggested by the prior art and thus, there is no basis for a *prima facie* case of non-obviousness.

Even if one were to assume that the Examiner has established a proper basis for a *prima facie* case, Applicants submit that a §103 rejection of the claims as presently amended to recite PNHX Na^+/H^+ transporters (hereafter "PNHX") would constitute a hindsight

reconstruction of the present invention. It is submitted that there are at least three lines of evidence to support Applicants' position of non-obviousness for the claims as presently amended; (1) The absence of intervening art disclosing PNHX introduced into plants; (2) The solution of a long felt need as recognized by professional journals; and (3) Unexpected results.

(1) The Absence of Intervening Art

Disclosing PNHX Introduced into Plants

The Young et al. application was filed in 1990. The Gordon-Kamm et al. publication relied upon by the Examiner was published in 1990 and is cited on page 10 of the Young et al. application. Despite the approximate nine year period between the filing of the Young et al. application and the filing of the present application, there is no indication in the publications cited by the Examiner that a PNHX was isolated and introduced into a plant. The failure to find a PNHX may be due to the confusion in the art as indicated in the enclosed Munns publication which reviews the progress in improving the salt tolerance of plants and indicates the confusion in the art as to how to proceed in identification of the gene(s) responsible for salt tolerance. ("Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses," *Plant, Cell and Environment* (1993)16:15-24.)

> As stated on page 15, second column, first full paragraph of Munn:
>
> "Physiologists can improve the salt tolerance of plants only by defining genes or characters for geneticists or breeders to exploit. However, we are still uncertain what enzymes or metabolic processes are important in salt tolerance, so molecular biologists are forced to search haphazardly for 'stress-induced proteins'. We are even uncertain whether the main mechanism of salt tolerance lies in the roots or the leaves, and whether it is in the growing or mature tissues, i.e., whether it is in the production or utilization of photosynthate, so it is not surprising that searches for 'stress-induced proteins' have so far been unsuccessful."

Instead of suggesting that plant equivalents to sod2 could be identified and introduced to plants, Young et al. focus on yeast homologs to sod2 suggesting that "Additional variants of the sod2 gene can be identified in other yeast species by hybridation screening." (See paragraph bridging the bottom of page 5 and the top of page 6 of Young et al.) Furthermore, the Hahnenberger et al. (1996) publication relied on by the Examiner in one of the §102 rejections includes as the other remaining authors Paul G. Young and Jia Zhengping. Paul G.

Young and Jia Zhengping appear to be the same two inventors identified in the Young et al. application. Despite its publication six years after the filing of the Young et al. application, the Hahnenberger et al. makes no mention of plant equivalents to sod2 nor is there any mention of successful introduction of sod2 into plants.

Applicants submit that given the importance of developing salt tolerant plants, if it would have been obvious to identify and introduce a PNHX into a plant given the disclosure of Young et al., it would have been done in the intervening nine-year period between the filing of Young et al. and the filing of the present application. Given the above, Applicants submit that the present invention as presently claimed is non-obvious in view of Young et al. and Gordon-Kamm et al.

(2) Solution of Long Felt Need and Professional Recognition of a Solution to this Long Felt Need

There has been a long felt need in the art for salt tolerant plants. The present invention satisfies this need by providing methods for producing salt tolerant plants. It should also be recognized that Applicants have demonstrated increased salt tolerance in *Arabidopsis*, which is a glycophytic plant with a sensitivity to salt similar to most crop plants. The fact that the present invention satisfies this long-felt need is supported by the recognition of the importance of the present invention in professional journals and the New York Times, the Washington Post, Science News, and NewScientist.com.

The importance of the present invention is demonstrated by the publication of the invention in the journal *Science* (Apse, M., Aharon, G., Snedden, W., Blumwald, E., "Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in *Arabidopsis*," *Science* (1999) 285:1256-1258). In addition to the recognition provided by publication in *Science*, the importance of the present invention was recognized in an article in *Chemical & Engineering News*, which discussed the *Science* article. In particular, Dr. Michael C. Shannon, head of the U.S. Salinity Laboratory, stated, "This is certainly one of the most interesting accomplishments in the field of salinity tolerance in the last two or three decades." ("Botanists design plants with a taste for salt," *Chemical & Engineering News*, (August 23, 1999)).

Subsequent research based on the present invention was published in *Nature Biotechnology* (Zhang, H.X. and Blumwald, E., "Transgenic salt tolerant tomato plants accumulate salt in foliage but not in fruit," *Nature Biotechnology* (2001) 19:765-768) demonstrating the effectiveness of the salt tolerance gene when expressed in a transgenic tomato plant. The importance of the present invention is further demonstrated by an article that appeared in the August 14, 2001 edition of the New York Times ("Altered Tomato Thrives in Salty Soil," by Anahad O'Connor). This article discusses the present invention as demonstrated in the *Nature Biotechnology* article. The New York Times article states that scientists have been trying for more than 50 years to produce salt tolerant crops and that it was not until Dr. Blumwald's invention that the real breakthrough came. The Washington Post article ("A New Strain of Tomatoes, and Don't Hold the Salt," July 31, 2001, page A03, copy of on-line version enclosed) also discusses the invention as demonstrated in the *Nature Biotechnology* article and states this advance "could help solve one of the biggest problems in agriculture." Similarly, the *Science News* article ("Gene makes tomatoes tolerate salt," *Science News*, vol. 160, August 4, 2001) comments on the *Nature Biotechnology* article, stating "Blumwald and a collcague have now made a dramatic advance ..." and, quotes plant biologist Edward Glenn, who stated "It's a really important breakthrough." Glenn, who studies halophytes at the University of Arizona is quoted as stating "Blumwald has made the key breakthrough that has been needed in salt tolerance for the past the last 30 years" in an article in *NewScientist.com* ("Gene-modified tomato revels in salty soils," July 31, 2001).

The fact that the present invention satisfies a long-felt need is demonstrated by publication of the invention in top-tier scientific journals and by comments in the press, indicating that the invention is a breakthrough in the field of salt tolerance.

Copies of the *Science*, *Nature Biotechnology* and *Chemical Engineering News* publications were provided in Applicants' Reply, dated October 12, 2001. Copies of the New York Times, Washington Post, Science News, and NewScientist.com article are enclosed.

(3) Unexpected Results

The present invention is also not obvious because of the unexpected results obtained. In particular, it was unexpected that overexpression of the AtNHX1 gene in a plant could

provide the dramatic increase in salinity tolerance disclosed in the present application. The Examiner is respectfully directed to pages 57 to 58 of the present application that describes the generation of transgenic Arabidopsis plants overexpressing AtNHX1. The transgenic plants were grown in different concentrations of NaCl as illustrated in Figure 7 (see caption on page 19 describing growth conditions for the plants).

Figure 7 shows that in contrast to wild-type plants, the transgenic plants grew in all of the salt concentrations tested. The ability of the transgenic plants to grow at high salt concentrations in contrast to wild-type plants is particularly well illustrated in Figure 7(e) and 7(f). Figures 7(e) and 7(f) show that at an NaCl concentration of 200 mM the transgenic plant grew to a level similar to the plant grown in 0 mM NaCl, while the wild-type plant grown in 200 mM NaCl was severely stunted in its growth.

In view of the above, Applicants submit that the invention as presently claimed is non-obvious.

III. The §112, First Paragraph, Rejections

A. The Examiner has rejected claims 1 to 14 and 17 to 32 under 35 U.S.C. §112, first paragraph. The Examiner has indicated that the specification, while being enabling for nucleic acids that encode SEQ ID NO:2, does not reasonably provide enablement for nucleic acids with homology to those nucleic acids or encode fragments of a transporter.

Applicants respectfully traverse the rejection. As discussed below, the specification provides guidance on how to isolate nucleic acid molecules that encode a PNHX transporter or fragments of a plant polypeptide having Na^+/H^+ transporter activity that provide increased salt tolerance in a cell. Applicants have also provided three examples of nucleic acids (AtNHX2-4) isolated and identified based upon homology to AtNHX1 (see page 56, line 8, to page 57, line 4 of the application) using the methods disclosed in the specification. Given the guidance in the specification, one of ordinary skill in the art could readily identify nucleic acid molecules that have homology to AtNHX1 and test their gene products for the ability to transport Na^+/H^+ ions and provide increased salt tolerance in a cell.

The Examiner has indicated that Applicants have failed to provide guidance to enable one to predict whether a nucleic acid, which is homologous to AtNHX1, has Na^+/H^+ transporter activity and can provide increased salt tolerance in a cell. Applicants assert that the amino acid sequence alignment of AtNHX1-3 of Figure 2b does provide adequate guidance of what protein regions are important for Na^+/H^+ transporter activity. For instance, based on this alignment one of skill in the art would recognize that amino acids 82-107 (FFIYLLPPIIFNAGFQVKKKQFFRNF), 147-196 (LAIGAIFAATDSVCTLQVLNQDETPL LYSLVFGEGVVNDATSVVVFNAIQ), and 256-325 (LMAYLSYMLAELFDLSGILTVFFC GIVMSHYTWHNVTESSRITTKHTFATLSFLAETFIFLYVGMDALDI) of AtNHX1 are highly conserved between AtNHX1-3. Such conserved regions of protein families have been used for over a decade to identify other functionally related genes. For example, Strathmann et al. (copy enclosed) teaches methods for the identification of functionally related genes by using degenerate PCR primers (Strathmann et al. (1991) *Proc. Natl. Acad. Sci. U.S.A.* 86:7407-7409). The degenerate PCR primers were designed using amino acid sequence alignments of known proteins. The sequence alignments indicated regions of homology and thus enabled the authors to isolate other functionally related genes which also contained these homologous regions. Other well known methods for identification of functionally related genes include screening cDNA and genomic libraries with nucleic acid probes and antibodies, and bioinformatics (see Example 2; page 44, lines 10-19)

Applicants also submit that it would not require undue experimentation on the part of one skilled in the art to readily identify nucleic acid molecules that have homology to AtNHX1 and test their gene products for the ability to transport Na^+/H^+ ions and provide increased salt tolerance in a cell. Claims 2 to 14 and 17 to 32 require that a nucleic acid must have homology (i.e., hybridize to a nucleic acid of SEQ ID NO:1) with AtNHX1, and must have Na^+/H^+ transporter activity that provides increased salt tolerance in a cell. The experimental methods used to determine homology between nucleic acid sequences and proteins are mentioned above and have been well established and routinely used for over a decade. Using the methods described in Examples 3 to 6 and 8 to 11 on pages 44 to 55 of the application, one of skill in the art would be able to introduce a nucleic acid sequence homologous to AtNHX1 into plants, plant cells, or yeast cells and determine if the sequence

encodes for a gene product that has Na^+/H^+ transporter activity and provides increased salt tolerance in a cell. Thus, Examiner's example of a urea transporter would fail to satisfy the requirement that the protein must have the ability to transport Na^+/H^+ ions and provide increased salt tolerance in a cell.

In the present Office Action the Examiner refers to pages 3 to 6 of the Office Action of April 12, 2001. On these pages the Examiner cited Bowie et al., Lazar et al., Broun et al., and Bork, which relate to predicting how amino acid changes will effect a protein's function. Applicants submit that these publications are irrelevant since the present invention requires a nucleic acid to have Na^+/H^+ transporter activity that provides increased salt tolerance to a cell.

In the present Office Action the Examiner refers to pages 3 to 6 of the Office Action of April 12, 2001. On page six of this action the Examiner states that "undue experimentation would have been required by one skilled in the art to develop and evaluate nucleic acid with 17% sequence similarity to AtNHX1 or that hybridizes to part of SEQ ID NO:1". Applicants submit that the proper standard for a §112, first paragraph, enablement rejection is whether the application contains sufficient information regarding the subject matter of the claims as to enable one skilled in the pertinent art to make and use the claimed invention without undue experimentation. MPEP §2164.01. Accordingly, the issue at hand is whether one of skill in the art having the present specification before him could make and use the claimed invention without undue experimentation. With regard to identifying nucleic acid sequences and proteins that have homology to AtNHX1 and isolation of those sequences, the Examiner is directed to Figure 2b of the application, which illustrates an amino acid sequence alignment that distinctly points out regions of homology; and Example 2 of the application, which discloses methods for identification of functionally related genes including bioinformatics, screening cDNA and genomic libraries with nucleic acid probes and antibodies, and PCR techniques using degenerate primers. With regard to determining whether a nucleic acid encodes a Na^+/H^+ transporter, the Examiner is directed Example 8 on pages 49 to 51 of the application, which discloses methods for introducing nucleic acids into plants, plant cells and yeast followed by determination if the nucleic acid encodes for a Na^+/H^+ transporter by measuring its activity. The methods for measuring Na^+/H^+ transporter activity include, but are not limited to, measurement of intracellular pH using fluorescein-derived compounds and ^{22}Na

influx and have been in use by those skilled in the art since the 1980s. Furthermore, the Examiner is directed to Examples 3 to 6 and 9 to 11 on pages 44 to 55 of the application, which discloses methods determining if expression of the nucleic acid confers salt tolerance on the plants, plant cells or yeast.

Given the guidance in the application as identified above and the level of skill in the art, Applicants submit that the claimed invention would not require undue experimentation. As stated in the MPEP, section 2164.06:

> "[A]n extended period of experimentation may not be undue if the skilled artisan is given sufficient direction or guidance." *In re Colianni,* 561 F.2d 220, 224, 195 USPQ 150, 153 (CCPA 1977). " 'The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.' " *In re Wands,* 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) (citing *In re Angstadt,* 537 F.2d 489, 502-04, 190 USPQ 214, 217-19 (CCPA 1976)).

Given the guidance in the Specification, the Examples section of the application, and methods known in the art, any experimentation necessary to determine whether a nucleic acid has homology (i.e., will hybridize to a nucleic acid of SEQ ID NO:1) with AtNHX1, and has Na^+/H^+ transporter activity that provides increased salt tolerance in a cell would be routine for one skilled in the art.

In view of the above, Applicants request respectfully that the Examiner reconsider and withdraw the §112, first paragraph rejection of claims 1 to 14 and 17 to 32.

B. The Examiner has rejected claims 1 to 14, 17 to 32, and 53 to 54 under 35 U.S.C. §112, first paragraph, as containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor(s) had possession of the claimed invention. The Examiner also refers to the arguments provided on pages 6 and 7 of the Office Action of April 12, 2001.

Applicants respectfully traverse the rejection. The "Guidelines for Examination of Patent Applications under the 35 U.S.C. §112, ¶1, 'Written Description' Requirement" (66

Fed. Reg., Vol. 66, No. 4p, 1099-1111 (Jan. 5, 2001)) identify the factors to be analyzed to determine if an inventor had possession of a claimed invention. The factors include 1) the level of skill and knowledge in the art, 2) complete or partial structure, 3) physical and/or chemical properties, 4) functional characteristics, 5) the correlation between structure and function, and 6) the method of making the claimed invention. Furthermore, the guidelines indicate that combinations of any of these characteristics can demonstrate possession of the claimed invention. Applicants submit that in view of the above factors, the written description requirement has been met.

With regard to the level of skill and knowledge in the art, Applicants submit that there are well established techniques in the art for identifying genes and the functions provided by the polypeptides encoded by these genes. Given these techniques and the guidance in the application, one of skill in the art could readily identify putative plant Na^+/H^+ transporters and test these putative transporters for the ability to provide salt tolerance. Applicants have demonstrated, using standard techniques, that they could isolate multiple plant Na^+/H^+ transporters capable of providing salt tolerance for a cell. Given the disclosure in the application of the nucleic acid and amino acid sequences of AtNHX1 and the disclosed techniques for identifying Na^+/H^+ transport and salt tolerance, one of skill in the art would conclude applicants were in possession of the claimed invention.

With regard to complete or partial structure, Applicants have provided information on the structure of embodiments of the claimed subject matter in the form of DNA/amino acid sequences for AtNHX1-4.

With regard to physical and/or chemical properties, Applicants have identified the property of Na^+/H^+ transport for the claimed subject matter.

With regard to functional characteristics, Applicants have identified the functional characteristic of salt tolerance.

With regard to correlation between structure and function, applicants have illustrated the correlation between structure and function by identifying various conserved regions in

proteins (AtNHX1-4) that are Na^+/H^+ transporters that provide salt tolerance. For example the polynucleotide and polypeptide sequences of AtNHX1-4 are provided and the sequence alignment in Figure 2b of the application identifies common structural features of the genus by indicating those regions of homology that are conserved in the AtNHX1-3 proteins. Accordingly, applicants have identified common structural features of the claimed plant Na^+/H^+ transporters as embodied by AtNHX1-4 and have identified properties and functional characteristics for these proteins.

With regard to methods of making the invention, the methods of making the invention are clear and enabling to one of skill in the art. As mentioned above, methods of using conserved regions of a protein family to identify other functionally related proteins have been used for over a decade. Molecular biological methods such as PCR, library screening, cloning, transfecting, and measurement of Na^+/H^+ transporter activity are routine. The identification of AtNHX2-4 based upon the sequence of AtNHX1 proves that methods of the application are valid and that applicants possessed a variety of plant Na^+/H^+ transporters.

Applicants submit that the combination of the characteristics described above demonstrate that Applicants were in possession of the claimed invention. Furthermore, the Examiner's assertion that inventors have not provided guidance as to how to alter SEQ ID NO. 1 to produce an Na^+/H^+ transporter versus other transporters does not meet the burden of presenting evidence or reasons why one of skill in the art would recognize that Applicants were not in possession of plant Na^+/H^+ transporters capable of providing salt tolerance for a cell.

C. The Examiner has rejected claim 1 under 35 U.S.C. §112, first paragraph as containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor(s) had possession of the claimed invention because the phrase "not the sequence having GenBank Accession No. AF007271" is not found anywhere in the specification.

Applicants have amended claim 1 of the present application to delete references to "GenBank Accession No. AF007271" and instead recite "wherein said nucleic acid molecule

is not the sequence of the gene A_TM021B04.4 or complementary to all of the sequence of the gene A_TM021B04.4". The gene A_TM021B04.4 is defined as the complement of nucleotides 65405 to 68024 of GenBank Accession No. AF007271 (see page 6 of Dante et al.; copy of pages 1, 6, 26, and 27 enclosed). In the Office Action of December 3, 2001, the Examiner provided a copy of a sequence alignment which identifies the sequence and sequence numbering of the A_TM021B04.4 gene. Support for this amendment can be found on page 21, lines 15 to 22, of the application, specifically the sentence bridging page 21, lines 17 to 18, which states, "A comparison of this full length cDNA with the *Arabidopsis* genome sequence (A_TM021B04.4) revealed the presence of 13 introns and 14 exons".

In view of the above, Applicants request respectfully that the Examiner reconsider and withdraw the §112, first paragraph rejection of claims 1 to 14, 17 to 32, and 53 to 54.

IV. The §112, Second Paragraph, Rejection

The Examiner has rejected claims 5 and 12 to 14 under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the invention.

Applicants have amended claim 5 to recite "that provides increased salt tolerance in a cell" instead of "and capable of increasing salt tolerance in a cell". This amendment reflects a change in wording and as such does not constitute new matter.

Applicants have amended claim 12 to recite "the cytosol of a first cell transformed with the nucleic acid molecule of any of claims 1 to 4 to provide the first cell with increased salt tolerance relative to a second non-transformed cell". Support for this amendment may be found in the legend for Figure 7, found on page 18, line 24 to page 19, line 24, of the application as well as in Figures 7(a) and 7(b), which disclose that *A. thaliana* plants comprising cells overexpressing a PNHX show increased salt tolerance as compared to wild-type *A. thaliana* plants. As this amendment provides a standard for ascertaining the degree of

salt tolerance, Applicants request that the rejection under 35 U.S.C. §112, second paragraph, of claim 12 and claims dependent thereon be withdrawn.

Applicants have amended claim 13 to reflect the change in language of claim 12 from which it depends.

In view of the above, Applicants request respectfully that the Examiner reconsider and withdraw the §112, first paragraph rejection of claims 5 and 12 to 14.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

Enclosed are two Revocation of Prior Power of Attorney documents (which include a new Power of Attorney). One document was executed by inventors Blumwald, Apse and Aharon, and the second executed by inventor Snedden. Also enclosed herewith is a Petition for extension of time to respond to the Examiner's Action and a Notice of Appeal. The Commissioner is hereby authorized to charge the fees associated with this communication to Deposit Account No. 19-5425.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims

1. (Twice amended) An isolated nucleic acid molecule encoding a PNHX transporter polypeptide[,] or a fragment of a plant polypeptide having Na^+/H^+ transporter activity that provides increased salt tolerance in a cell, wherein said nucleic acid molecule is not the sequence of the gene A_TM021B04.4 or complementary to all of the sequence of the gene A_TM021B04.4[having GenBank Accession No. AF007271].

2. (Twice amended) An isolated nucleic acid molecule encoding a [TNHX transporter polypeptide,]PNHX transporter polypeptide[,] or a fragment of a plant polypeptide having Na^+/H^+ transporter activity that provides increased salt tolerance in a cell, wherein said nucleic acid molecule is not the sequence of the gene A_TM021B04.4 or complementary to all of the sequence of the gene A_TM021B04.4, comprising a nucleic acid molecule selected from the group consisting of:

(a) a nucleic acid molecule that hybridizes to all or part of a nucleic acid molecule shown in [SEQ ID NO:1], or a complement thereof under moderate or high stringency hybridization conditions, wherein the nucleic acid molecule encodes a [TNHX transporter polypeptide, a]PNHX transporter polypeptide or a plant polypeptide having Na^+/H^+ transporter activity and capable of increasing salt tolerance in a cell;

(b) a nucleic acid molecule degenerate with respect to (a), wherein the nucleic acid molecule encodes a [TNHX transporter polypeptide, a] PNHX transporter polypeptide or a plant polypeptide having Na^+/H^+ transporter activity and capable of increasing salt tolerance in a cell.

4. (Twice amended) An isolated nucleic acid molecule encoding a [TNHX transporter polypeptide or a]PNHX transporter polypeptide[,] or a fragment of a plant polypeptide having Na^+/H^+ transporter activity and that provides increased salt tolerance in a cell, wherein said nucleic acid molecule is not the sequence of the gene A_TM021B04.4 or complementary to all of the sequence of the gene A_TM021B04.4, comprising a nucleic acid molecule selected from the group consisting of:

(a) the nucleic acid molecule of the coding strand shown in [SEQ ID NO:1], or a complement thereof;

(b) a nucleic acid molecule encoding the same amino acid sequence as a nucleotide sequence of (a); and

(c) a nucleic acid molecule having at least 30% identity with the nucleotide sequence of (a) and which encodes a [TNHX transporter or the]PNHX transporter polypeptide or a plant polypeptide having Na^+/H^+ transporter activity.

5. (Amended) The nucleic acid molecule of any of claims 1 to 4, wherein [the THX transporter polypeptide or]the PNHX transporter polypeptide comprises an AtNHX transporter polypeptide having Na^+/H^+ transporter activity that provides increased [and capable of increasing]salt tolerance in a cell.

7. (Twice amended) An AtNHX nucleic acid molecule isolated from *Arabidopsis thaliana*[,] or a fragment thereof encoding a transporter polypeptide having Na^+/H^+ transporter activity that provides increased salt tolerance in a cell, wherein said nucleic acid molecule is not the sequence of the gene A_TM021B04.4 or complementary to all of the sequence of the gene A_TM021B04.4.

12. (Twice amended) The nucleic acid molecule of any of claims 1 to 4, wherein [the TNHX transporter polypeptide or]the PNHX transporter polypeptide extrudes monovalent cations out of the cytosol of a first cell transformed with the nucleic acid molecule of any of claims 1 to 4 to provide the first cell with increased salt tolerance relative to a second non-transformed cell, wherein the monovalent cations are selected from at least one of the group consisting of sodium, lithium and potassium.

13. (Amended) The nucleic acid molecule of claim 12, wherein the cells[cell] comprise[s a] plant cells[cell].

27. (Amended) A method of producing a genetically transformed plant which expresses [TNHX or]PNHX transporter polypeptide, comprising regenerating a genetically transformed plant from the plant cell, seed or plant part of claim 21.

28. (Twice amended) The method of claim 26, wherein the genome of the host cell also comprises a functional [TNHX or]PNHX gene.

29. (Twice amended) The method of claim 26, wherein the genome of the host cell does not comprise a functional [TNHX or]PNHX gene.

31. (Twice amended) A method for expressing a [TNHX or]PNHX transporter polypeptide in the host cell of claim 19, the method comprising culturing the host cell under conditions suitable for gene expression.

53. (Twice amended) A method of producing a genetically transformed plant which expresses or overexpresses [a TNHX transporter polypeptide,]a PNHX transporter polypeptide or a plant polypeptide having Na^+/H^+ transporter activity and provides increased salt tolerance in a cell, wherein said nucleic acid molecule is not the sequence of the gene A_TM021B04.4 or complementary to all of the sequence of the gene A_TM021B04.4, and wherein the plant has increased salt tolerance, comprising:

(a) cloning or synthesizing [a TNHX nucleic acid molecule,]a PNHX nucleic acid molecule or a nucleic acid molecule which codes for a plant Na^+/H^+ transporter polypeptide, wherein the polypeptide is capable of providing salt tolerance to a plant and wherein said nucleic acid molecule is not the sequence of the gene A_TM021B04.4 or complementary to all of the sequence of the gene A_TM021B04.4;

(b) inserting the nucleic acid molecule in a vector so that the nucleic acid molecule is operably linked to a promoter;

(c) inserting the vector into a plant cell or plant seed;

(d) regenerating the plant from the plant cell or plant seed, wherein salt tolerance in the plant is increased compared to a wild type plant.

56. (New) An isolated nucleic acid molecule encoding a TNHX transporter polypeptide or a PNHX transporter polypeptide, or a fragment of a polypeptide having Na^+/H^+ transporter activity that provides increased salt tolerance in a cell, comprising [SEQ ID NO. 1].